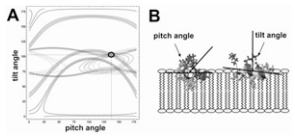
The N-terminal domain of huntingtin (Htt17), located immediately upstream of the decisive polyglutamine tract, strongly influences important properties of this large protein and thereby the development of Huntington's disease. Htt17 markedly increases polyglutamine aggregation rates and huntingtin's interactions with biological membranes. Here, an ensemble of low-energy conformations of the protein domain was identified by solution NMR in interfacial environments, and the structure was further refined using solid-state NMR spectroscopy on oriented phospholipid bilayers. The pronounced structural transitions of Htt17 upon membrane-association result in an in-plane aligned α-helical conformation from K6 to F17. The membrane binding of Htt17 and the resulting permeability were quantitatively analyzed and are strongly dependent on lipid composition, whereas the helical tilt angle (~77 degrees) is nearly constant in all membranes investigated. The structure and lipid interactions of Htt17 have pivotal implications for membrane-anchoring and functional properties of huntingtin and concomitantly the development of the disease.




The Figure shows the solid-state NMR orientational restraints from three 15N and one 2H labelled sites (A) and the resulting alignment of the solution NMR structure in the lipid bilayer (B).

**1859-Plat**
**Investigating the Mechanism by which Bcl-xL Regulates Ceramide Channels**
**Kai-Ti Chang**, Justin Wang, Marco Colombini.
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The level of ceramide, a sphingolipid, increases in mitochondria early in apoptosis resulting in the formation of ceramide channels. These channels are involved in the release of intermembrane space proteins, such as cytochrome c, into the cytosol. This release is a crucial and irreversible step in the apoptotic process. Formation of ceramide channels is inhibited by Bcl-xL, an antiapoptotic protein. Insights into the molecular basis for this regulation were obtained in a study of ceramide analogs (Perera, M. N. et al., Biochem. J. 445, 81, 2012). The results indicated that the effectiveness of Bcl-xL is very sensitive to changes in the hydrophobic regions of the ceramide channel. Furthermore, inhibitors (ABT- 737, ABT-263 and antimycin A) that specifically bind to the hydrophobic groove of Bcl-xL interfere with this function of Bcl-xL. These results imply that the hydrophobic groove is important for Bcl-xL to inhibit channel formation or dissemble the channel. We have demonstrated direct binding of a ceramide molecule to Bcl-xL by a fluorescent ceramide competition technique. In addition, site-directed mutagenesis at a single residue in multiple locations in the hydrophobic groove has resulted in a reduction of the inhibitory action of Bcl-xL. These results support the conclusion that Bcl-xL regulates the ceramide channel through the hydrophobic pocket. This same feature is critical to the regulation of proapoptotic Bcl-2 family proteins, thus it inhibits comprehensively all the pro-apoptotic processes of the cell. (supported by NSF grant MCB-1023008)

**1860-Plat**
**Elucidating the Molecular Details of Phosphatidylserine Membrane Recognition in Immune Response**
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The immune system recognizes a vast array of chemical signatures as antigens although historically most research has focused more exclusively on protein/protein recognition. More recently it has been appreciated that lipid membranes can also provide important immunological signals as demonstrated in both phosphatidylserine (PS) recognition in apoptotic cell clearance and transient PS exposure in T Cell activation. Despite the clear immunological importance of PS exposure and recognition, there remain very few molecular details regarding the mechanisms of PS membrane recognition. Even more fundamentally, it remains unclear if all PS exposing membranes are immunologically equal or if there exists a sensitivity to additional membrane properties beyond simply the presence or absence of PS. To address this gap in our understanding we have made use of a novel combination of biophysical and biochemical techniques to elucidate the molecular mechanisms by which Tim4 (an immune related PS receptor) recognizes PS containing membranes. Tryptophan fluorescence binding assays have revealed that Tim4 binding is sensitive to membrane PS composition suggesting that there is more to the story than a single PS to single protein interaction. By utilizing a combination of x ray reflectivity measurements to determine membrane bound protein orientation and depth of penetration, as well as molecular dynamics simulations to support the experimental results, we have developed a protein/membrane binding model that provides structural evidence to explain the unique complexities of Tim4 mediated PS membrane recognition. Most significantly, these results provide a standard against which other immunologically related PS receptors can be compared, thereby allowing us to begin to address the more fundamental question of just how important lipid membrane recognition is for our bodies' immunological defense mechanisms.

**1861-Plat**
**Investigating the Molecular Basis of cPLA2α Membrane Bending**
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Signal transduction mediates disease through key molecular targets that initiate signaling networks. As protein-lipid interactions have been examined in the literature, their role in cellular signaling has become more prevalent as lipid-binding proteins have become high impact drug targets in cancer, inflammation and viral egress. One such target, termed cytosolic phospholipase $A_2$ α (cPLA$_2$α), has been shown to play a key role in the production of the inflammatory mediators prostaglandins and leukotrienes. A novel function of the protein was recently discovered in our lab showing cPLA$_2$α bends zwitterionic membranes using model membranes, a process that is mediated by cPLA$_2$α's ability to deeply penetrate membranes. Others in the field have reported cPLA$_2$α to participate in Fc mediated phagocytosis, intra-Golgi trafficking and endosomal trafficking which further supports cPLA$_2$α's ability to bend membranes in biological processes. In addition, direct evidence has been reported using siRNA showing that cPLA$_2$α induced vesiculation in cells. These results translate into our cellular system as cells transfected with eGFP-cPLA$_2$α form cytoplasmic vesicular structures. We have preliminary evidence showing cPLA$_2$α membrane bending is mediated by oligomerization. The origin of oligomerization is currently under further investigation using both *in vitro* and cellular techniques.

**1862-Plat**
**Probing for π-Cation Interactions in the Binding of B. Thuringiensis Phosphatidylinositol-Specific Phospholipase C Phosphatidylcholine-Rich Vesicles**
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Bacillus thuringiensis phosphatidylinositol specific phospholipase C (PI-PLC) binds tightly to phosphatidylcholine (PC)-rich vesicles. A possible mechanism for tight binding to PC interfaces involves tyrosine π / choline cation complexes. With this in mind, we have mutated surface tyrosine residues (Y86A, Y88A, Y204S, Y246A, Y247A, Y248A, Y251A), located on the barrel rim and in two helices of this (αβ)-barrel protein, to assess their contribution to vesicle binding. None of these mutations significantly alter the rate of PI cleavage in vesicles, as long as the PI concentration is $> 4$ mM. However, binding to PC-containing vesicles, as measured by fluorescence correlation spectroscopy, showed a loss of affinity. The loss-of-Tyr mutant proteins fall into two classes: (i) those where Kd(mut)/Kd(WT) $< 5$ (Y86A, Y247A) and (ii) those where the ratio of mutant Kd to that of the WT was 100-300 (Y88A, Y204S, Y246A, Y248A, Y251A). With the exception of Y204S/Y251A the effects of the mutations appear to be additive, We also attempted to enhance interactions with PC by introducing new Tyr or Trp residues on the surface, but these mutations either reduced membrane affinity or left it unchanged. Apparently, more specific interactions are needed to enhance binding. Estimating ΔΔG for these Tyr/PC interactions from the apparent Kd values, we find that the free energy associated with Tyr86 and Tyr247 is ~ 4 kJ/mol, comparable to the value predicted by the Wimley-White scale. In contrast, removal of the other surface Tyr is linked to a higher energy cost: 10-13 kJ/mol towards pure PC vesicles. These higher energies

may indicate π-cation interactions using two or more Tyr residues with the PC headgroup.

**1863-Plat**
**Lipid Bilayer Influences Rhodopsin Activation Probed by FTIR and UV-Visible Spectroscopy**
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Rhodopsin, the mammalian dim light photoreceptor, is the model protein for studying G protein-coupled receptor (GPCR) structure and function. We postulate that this process can be attributed to an ensemble of activated states - the ensemble activation mechanism (EAM) - and that the EAM is directly affected by membrane protein-lipid bilayer interactions dependent upon cell membrane composition, as predicted by the flexible surface model [1]. Rhodopsin was detergent-solubilized and incorporated into homogenous lipid vesicles (either POPC or DOPC) by dialysis. Samples were prepared at a series of temperature and pH values, bleached, and analyzed by simultaneous Fourier transform infrared (FTIR) and UV-visible spectral acquisition. Activation in mixed-chain POPC bilayers drastically backshifts rhodopsin from the active Meta II photointermediate to the inactive Meta I state. A di-monounsaturated phospholipid like DOPC restores partial activity to rhodopsin, as well as stabilizing the protein in the Meta IIa state. Spectral reduction and analysis via pH titration curves yielded non-Henderson-Hasselbach behavior, indicating more than one activated state exists, thus supporting the concept of an EAM [2]. In addition, temperature changes have a marked effect on rhodopsin activation, decoupling the two protonation switches necessary for full activation at physiological temperatures. By manipulating the lipid environment, we validate the flexible surface model and EAM. Lipids with negative monolayer curvature such as DOPC facilitate rhodopsin activation towards the Meta II state [3]. Thermodynamic parameters showed that free energy changes are related to greater flexibility of rhodopsin in the cell membrane upon activation. Further validation of the flexible surface model is an important contribution to biophysical understanding of GPCR function.
[1] A.V. Botelho et al. (2006) BJ 91, 4464-4477. [2] M. Mahalingam et al. (2008) PNAS 105, 17795-17800. [3] E. Zaitseva et al. (2010) JACS 132, 4815-4821.

**1864-Plat**
**Membrane Curvature Elastic Stress Strongly Modulates Metarhodopsin II Formation**
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We studied the influence of curvature elastic stress in lipid monolayers induced by hydrophobic mismatch between lipid bilayer thickness and the hydrophobic length of rhodopsin transmembrane helices on the metarhodopsin I (MI)/metarhodopsin II (MII) equilibrium. Experiments were conducted at the low rhodopsin/lipid ratio of 1/1,000 to suppress rhodopsin oligomerization. Elastic stress was generated by reconstituting dark-adapted rhodopsin into a series of phosphatidylcholine (PC) bilayers with acyl chains of 14-20 carbons in length and cholesterol content varied from 0 to 30 mol %. $^2$H NMR was used to monitor the adjustment of the length of hydrocarbon chains to the protein and to characterize monolayer curvature at the protein-lipid interface. We found that the average length of hydrophobic regions on rhodopsin transmembrane helices is 2.6 ± 0.1 nm. Thinner bilayers stretch and bend with negative monolayer curvature to match the length of hydrophobic helices, while thicker bilayers get thinner with positive monolayer curvature near the protein. Shifts in the MI/MII equilibrium depended on the sign of monolayer curvature: negative curvature favored MI while positive curvature favored MII. The results suggest that MII formation generates negative curvature in monolayers near the protein, thus raising curvature stress for thin bilayers but releasing stress for thicker bilayers. Addition of cholesterol increases bilayer hydrophobic thickness, but otherwise showed identical behavior: bilayers with a hydrophobic thickness less than 2.6 nm favored MI while thicker bilayers favored MII. In case of severe hydrophobic mismatch between rhodopsin and bilayers, the behavior was complicated by rhodopsin oligomerization that seems to favor MI. The sensitivity of the MI/MII equilibrium to negative curvature elastic stress was observed earlier in experiments with phosphatidylethanolamines by us and the Brown laboratory. Negative curvature stress upon MII formation is critical for understanding shifts in the MI/MII equilibrium.

# Platform: Transcription

**1865-Plat**
**Backtracking and Other Off-Path Pauses Control the Dynamic of Viral RNA Dependent RNA Polymerases**
**David Dulin**[1], Igor D. Vilfan[2], Bojk A. Berghuis[1], Susanne Hage[1], Dennis H. Bamford[3], Minna Poranen[3], Martin Depken[1], Nynke H. Dekker[1].
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RNA-dependent RNA Polymerases (RdRPs) are key players in the transcription and replication of RNA viruses. We study P2 of the bacteriophage Φ6 as a model system for structurally similar polymerases of e.g. positive ssRNA viruses such as Hepatitis C. During the different phases of the viral life cycle, P2 RdRP alternately transcribes or replicates viral RNA. Transcription produces infectious positive-strand ssRNA whilst replication is the last step of the virus maturation in which the dsRNA genome is restored.
Benefiting from the parallelism afforded by magnetic tweezers, we simultaneously measure the transcription activity on tens of tethers while maintaining a resolution of 6 bases. In this way, we report on polymerase dynamics based on the analysis of an unusually large dataset (800 traces) taken under differing conditions of force and nucleotides concentration. This enables us to characterize P2 transcription elongation dynamics with unprecedented precision. Fits of this data to a kinetic model for polymerase activity via Maximum Likelihood estimation (MLE) reveal that the translocation step is insensitive to force and points to the existence of several types of off-pathway pause states in which the polymerase may be trapped. The associated pauses from short-lived exponentially-distributed pauses to long-lived pauses that follow a power law distribution over three decades and likely result from backtracking.
By comparing our single-molecule data to the results of previous structural and biochemical studies on related RdRPs, we propose that the exponentially-distributed pauses are connected to the nucleotide selection process. Backtracking has not been previously observed for the P2 RdRP, and our experiments show how it can be problematic for an RdRP, e.g. preventing it from completing transcription or replication.

**1866-Plat**
**A Quantitative Kinetic Model of Eukaryotic Transcription Elongation from Single-Molecule Experiments**
**Manchuta Dangkulwanich**[1], Toyotaka Ishibashi[1], Shixin Liu[1], Maria L. Kireeva[2], Lucyna Lubkowska[2], Mikhail Kashlev[2], Carlos Bustamante[1].
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Transcription by RNA polymerase II (Pol II) is an important point of control for eukaryotic gene expression, and has been extensively studied by structural, biochemical, and biophysical methods. During transcription elongation, Pol II moves processively along the DNA template and synthesizes RNA, one nucleotide at a time. A comprehensive kinetic characterization of this process, the transcription elongation cycle, incorporating both the on-pathway nucleotide addition phase and the off-pathway pausing phase, is still lacking. We used an optical trapping assay to follow transcription by individual yeast Pol II on bare or nucleosomal DNA. The single-molecule technique employed here allowed us to separate analyze the nucleotide addition phase and pausing phase separately, and arrived at a kinetic model that quantitatively describes both phases of transcription elongation. This model was used to measure the effects of a point mutation in the trigger loop motif on the on- and off-pathway dynamics of transcription, and can serve as a general framework to study the roles of various transcription and chromatin remodeling factors in transcription.

**1867-Plat**
**Computational Studies on Physical Mechanisms of T7 RNA Polymerase Elongation and Nucleotide Selection**
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Beijing Computational Science Research Center, Beijing, China.
The RNA polymerase (RNAP) of bacteriophage T7 is a single subunit enzyme that can transcribe DNA to RNA in the absence of additional protein factors. In this work, we study T7 RNAP as a model system for transcription elongation. Based on structural information and experimental data from single-molecule force measurements, we had shown that a small translocation free energy bias aids initial nucleotide selection during elongation [1]. The selection is conducted by a conserved residue Tyr639 next to the active site. At the